REMARKS/ARGUMENTS

Claims 1-68 are pending in this application, and claims 5, 18, 31, 36, 49, 62 and 64-67 have been withdrawn pursuant to a Restriction Requirement/Species Election. Claims 1 and 33 have been amended. Claims 4 and 68 have been canceled without prejudice or disclaimer. Support for the amendments to the claims is found, *inter alia*, throughout the specification as originally filed. More particularly, support for the amendments to claims 1 and 33 is found, *inter alia*, in claim 4 as originally filed, and in Example I on pages 53-62 of the specification. No new matter has been introduced with the foregoing amendment. Reconsideration is respectfully requested.

Claims 1-3, 9, 33-34, 40 and 42 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Claim 68 has been rejected under 35 U.S.C. §§ 101 and 112, second paragraph. Claims 1-4, 6-15, 17, 19-22, 27-30, 32-35, 37-46, 48-53, 58-61 and 63 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious. Claims 1-4, 6-15, 17, 19-22, 27-30, 32-35, 37-46, 48-53, 58-61 and 63 have been rejected under the judicially created doctrine of obviousness-type double patenting. For the reasons set forth herein, each of the Examiner's concerns is overcome.

I. The Invention

The present invention provides novel and surprisingly effective methods for delivering nucleic acids to cells. These methods are based upon the discovery that the presence of endosomal membrane destabilizers, such as Ca^{++} ions, leads to a dramatic increase in the transfection efficiency of nucleic acids (*e.g.*, plasmids) formulated as nucleic acid-lipid particles (*e.g.*, SPLPs or "stabilized plasmid-lipid particles").

II. Rejection of Claims 1-3, 9, 33-34, 40 and 42 Under 35 U.S.C. § 112, Second Paragraph

Claims 1-3, 9, 33-34, 40 and 42 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. In making this rejection, the Examiner has indicated that the recitation of "said particle" lacks antecedent basis because "a nucleic acid-lipid particle composition" is not the same as the "particle."

In order to expedite prosecution of the present case, claims 1 and 33 have been amended to recite more clearly a nucleic acid-lipid particle composition comprising: (a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles and a nucleic acid; and (b) an endosomal membrane destabilizer, wherein said endosomal membrane destabilizer is Ca^{++} ion. In view of the amendment to claims 1 and 33, the Examiner concern is overcome. Accordingly, Applicants urge the Examiner to withdraw the rejection under 35 U.S.C. § 112, second paragraph.

III. Rejection of Claim 68 Under 35 U.S.C. § 112, Second Paragraph, and § 101

Claim 68 has been rejected under 35 U.S.C. § 112, second paragraph, and under 35 U.S.C. § 101. In order to expedite prosecution of the present case, claim 68 has been cancelled without prejudice or disclaimer. In view of the cancellation of claim 68, the Examiner's concern is overcome. Accordingly, Applicants urge the Examiner to withdraw the rejection under 35 U.S.C. §§ 112, second paragraph, and 101.

IV. First Rejection Under 35 U.S.C.§ 103

Claims 1-4, 6-15, 17, 19-22, 27-30, 32-35, 37-46, 48-53, 58-61 and 63 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious over U.S. Patent No. 5,705,385 ("Bally *et al.*"), taken with either PCT Publication No. WO 98/19710 or U.S. Patent No. 6,177,274 ("Park *et al.*"), and further in view of either Haberland *et al.*, *Biochemica et Biophysica Acta*, 1445, 21-30, April 14, 1999 ("Haberland *et al.*"), or U.S. Patent No. 6,270,761 ("Russell *et al.*"). To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

As set forth in M.P.E.P. § 2143,

> [t]o establish a *prima facie* case of obviousness, three basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves of in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed

> combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure.
>
> *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)

Applicants state that there is simply no motivation or suggestion provided in the cited references to modify their teaching in the way the Examiner has contemplated. Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion or motivation to do so found either in the reference itself or in the knowledge generally available to one of ordinary skill in the art. *In re Fine*, 837 F.2d 1071, 4 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

Claim 1 has been amended to clarify that the endosomal membrane destabilizer is Ca^{++} ion. Amended claim 1 reads as follows:

> Claim 1 (currently amended): A nucleic acid-lipid particle composition for introducing a nucleic acid into a cell, said particle composition comprising:
>
> (a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, a nucleic acid; and
>
> (b) an endosomal membrane destabilizer, wherein said endosomal membrane destabilizer is Ca^{++} ion.

Bally *et al.* teach an SPLP comprising DODAC/DOPE and PEG-ceramide wherein the nucleic acid is encapsulated in the lipid (*see*, columns 7-9, 11, and 13, Bally *et al.*). In addition, Bally *et al.* disclose that polycationic agents, such as polylysine or salts, can be added to the preformed particle in order to enhance the transfection of the particle to a cell of interest. Bally *et al.* also disclose that PEG or known derivatized PEG-lipids prevent particle aggregation and provide a means for increasing circulation lifetime, thereby increasing the delivery of the lipid-nucleic acid particles to target tissues. Bally *et al.* also disclose that the polynucleotide can be a nucleic acid construct encoding a therapeutic protein, that the average size of the preformed liposome is typically between about 100 nm and several microns, the

preferred molecular weight for PEG is about 1000 daltons, and that between 1-15 mole percent of such a derivatized lipid is included in the liposome formulation.

The Examiner correctly acknowledges that Bally *et al.* do ***not*** teach the incorporation of polylysines into the PEG-lipid conjugate, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP compositions of the present invention, e.g., either inside and/or outside of the SPLP (*see*, page 4 of the Office Action).

Park *et al.* teach the use of poly-L-lysine (PLL) as a targeting moiety and a DNA condensate (*see*, column 5, lines 34-47, Park *et al.*). The polylysine used by Park *et al.* is a linear polymer with a MW of 20-30 K. In stark contrast, the present invention utilizes PEG that is a branched chain headgroup made of only a few lysine residues and having a MW of only a few hundred. Moreover, Park *et al.* do ***not*** teach or suggest the use of cationic Ca^{2+} ion as an endosomal disrupting agent in the claimed SPLP compositions.

According to the Office Action, one of skill in the art would have been motivated to employ a Ca^{++} ion containing salt in a particulate form in the presently claimed SPLP compositions because Russell *et al.* teach that calcium phosphate crystals, when complexed with a delivery vector or agents, enhance the transfection of a nucleic acid in a cell. Russell *et al.* teach compositions and methods for delivery of nucleic acids. However, in stark contrast to the present invention, the compositions of Russell *et al.* comprise retroviral vectors. Importantly, Russell *et al.* teach that the addition of $CaCl_2$ enhances the co-precipitation of retroviral vectors (*see*, column 8, line 16-62, Russell *et al.*). Russell *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid conjugates, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the claimed SPLP compositions. Retroviral vectors are entirely different from the SPLP particles of the present invention. The present invention does not involve the use of retroviral vectors. As such, one of skill in the art would not have been motivated to use cationic Ca^{2+} ion as an endosomal disrupting agent in the SPLP compositions based on the teachings of Russell *et al.*

The Office Action also alleges that Haberland *et al.* teach that Ca^{2+} ions are an efficient cofactor of polycation-mediated gene transfer by functioning as an endosomal

disrupting agent. Haberland *et al.* teach the enhancement of transfection by calcium in cases where the transfection is carried out using cationic proteins such as the nuclear protein H1, the polycation polylysines, and a number of other commercial transfection agents (see, page 23, last paragraph, and page 24, first column, Haberland *et al.*). However, Haberland *et al.* in fact ***teach away*** from enhancing transfection efficiency in cases where cationic liposomes such as Lipofectin and Lipofectamine are used (*see*, page 24, column 2, Haberland *et al.*).

It is well-settled that the prior art as a whole must be considered, including those references which ***teach away*** from the claimed invention. The Federal Circuit in *Dow Chemical Co.*, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988), stated:

> The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in light of the prior art. . . .Both the suggestion and expectation of success must be founded in the prior art, not in the applicant's disclosure.
>
> In determining whether such a suggestion can be fairly gleaned from the prior art, *the full field of the invention must be considered*: for the person of ordinary skill is charged with knowledge of the entire body of technological literature, *including that which might lead away from the claimed invention.* . . . Evidence that supports, rather than negates, patentability must be fairly considered.

As set forth in M.P.E.P. § 2144.05 III., second paragraph:

> A *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention. *In re Geisler*, 116 F.3d 1465, 1471, 43 USPQ2d 1362, 1366 (Fed. Cir. 1997)

Moreover, as set forth in M.P.E.P. § 2145 X.D.2.:

> It is improper to combine references where the references teach away from their combination.
> *In re Grasselli*, 713 F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983)

According to Haberland *et al.*, "the transfection efficiency of cationic liposomes, such as Lipofectin and Lipofectamine, cannot be reproducibly enhanced by Ca^{2+}" (*see*, page 28, column 1, second paragraph, Haberland *et al.*). As the Examiner is aware, the SPLP compositions of the present invention comprise a cationic lipid. As such, one of skill in the art would not have been motivated to use cationic Ca^{2+} ion as an endosomal disrupting agent in the SPLP compositions based on the teachings of Haberland *et al.* In fact, Haberland *et al.* ***teach away*** from the use of Ca^{2+} to enhance transfection efficiency of cationic liposomes, such as the SPLP compositions of the present invention. Therefore, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

V. Second Rejection Under 35 U.S.C.§ 103

Claim 1-4, 6-17, 19-30, 32-35, 37-48, 50-61 and 63 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious in view of U.S. Patent No. 5,705,385 ("Bally *et al.*") taken either with PCT Publication No. WO 98/19710 ("Schacht *et al.*") or U.S. Patent No. 6,177,274 ("Park *et al.*"), either Haberland *et al.* or Russell *et al.*, and in further view of U.S. Patent No. 6,287,591 ("Semple *et al.*"). To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

Claim 1 has been amended to clarify that the endosomal membrane destabilizer is Ca^{++} ion. Bally *et al.* teach an SPLP comprising DODAC/DOPE and PEG-ceramide wherein the DNA is encapsulated in the lipid portion of the SPLP (*see*, columns 7-9, 11, and 13, Bally *et al.*). In addition, Bally *et al.* disclose that polycationic agents, such as polylysine or salts, can be added to the preformed particle in order to enhance the transfection of the particle to a cell of interest. The Examiner acknowledges that the combined cited references of Bally *et al.* taken with either Schacht *et al.* or Park *et al.*, and either Haberland *et al.* or Russell *et al.*, do ***not*** teach that the derivatized lipid-PEG is a diacylglycerolyl based PEG, or that the conjugated lipid comprises a diacyglycerolyl based PEG-polylysine-targeting ligand. Moreover, the Examiner acknowledges that Bally *et al.* do ***not*** teach the incorporation of polylysines into the PEG-lipid conjugate, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the presently

claimed SPLP compositions, e.g., either inside and/or outside of the SPLP particle (*see*, page 4 of the Office Action).

Park *et al.* teach the use of poly-L-lysine (PLL) as a targeting moiety and a DNA condensate (*see*, column 5, lines 34-47, Park *et al.*). However, Park *et al.* do ***not*** teach or suggest the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP particle containing composition of the present invention.

Schacht *et al.* teach synthetic polymer-based carrier vehicles made by self-assembly of the nucleic acid with cationic polymer material so as to condense the nucleic acid and form a polyelectrolyte complex. The complex is then reacted with reactive hydrophilic polymer material which bonds to the complex forming a hydrophilic coating that stabilizes the complex and provides the outer protective steric shield. Membrane-disrupting agents are taught to enable DNA to gain access to the cytoplasm of cells. However, Schacht *et al.* do ***not*** teach or suggest the use of endosomal membrane-disrupting agents in SPLP compositions. Furthermore, Schacht *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid conjugates.

As discussed above in Section IV above, Russell *et al.* teach that the addition of $CaCl_2$ enhances the co-precipitation of retroviral vectors (*see*, column 8, line 16-62, Russell *et al.*). Russell *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid conjugates, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP compositions. Moreover, Haberland *et al.* ***teach away*** from enhancing transfection efficiency in cases where cationic liposomes are used in the transfection process (*see*, page 24, column 2, Haberland *et al.*). Therefore, neither Russell *et al.* nor Haberland *et al.* supplement the deficiencies of Bally *et al.*, Schacht *et al.* and Park *et al.*

Semple *et al.* teach a lipid-therapeutic agent particles containing a charged therapeutic agent encapsulated in lipid portion containing at least two lipid components including a protonatable or deprotonatable lipid such as an amino lipid and a lipid that prevents particle aggregation during lipid-therapeutic agent particle formation, such as a PEG-modified or

polyamide oligomer-modified lipid. Semple *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid conjugates, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP compositions. Therefore, Semple *et al.* do not supplement the deficiencies of Bally *et al.*, Schacht *et al.*, Park *et al.* Russell *et al.*, and Haberland *et al.* As such, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

VI. Obviousness-Type Double Patenting Rejection

Claims 1-4, 6-17, 19-30, 32-35, 37-48, 50-61 and 63 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 19-63 of U.S. Patent Application No. 09/553,639 ("Cullis *et al.*") taken with Haberland *et al.* or Russell *et al.* To the extent the rejection is applicable to the amended set of claims, Applicants respectfully traverse the rejection.

Claim 1 has been amended to clarify that the endosomal membrane destabilizer is Ca^{++} ion. According to the Office Action, the claims of Cullis *et al.* read on lipid-based drug formulations comprising an SPLP comprising a lipid conjugate comprising a diacyglycerolyl based PEG-polylysine-targeting ligand, wherein the polylysine comprises at least for consecutive lysine residues for use in enhancing the delivery of a bioactive agent to cells of interest. Clearly, Cullis *et al.* do ***not*** teach or suggest the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the claimed SPLP compositions.

The Office Action alleges that one of skill in the art would have been motivated to employ a Ca^{++} ion in the SPLP composition because Russell teaches that calcium phosphate crystals, when complexed with a delivery vector agent, enhance the transfection of a nucleic acid to a cell, and because Haberland *et al.* teach that Ca^{++} ion is an efficient cofactor of polycation-mediated gene transfer by functioning as an endosomal disrupting agent. However, Russell *et al.* teach that the addition of $CaCl_2$ enhances the co-precipitation of retroviral vectors (*see*, column 8, line 16-62, Russell *et al.*). Retroviral vectors are entirely different from the SPLP particles of the present invention. The present invention does not involve the use of retroviral vectors. Russell *et al.* do ***not*** teach or suggest the incorporation of polylysines into the PEG-lipid

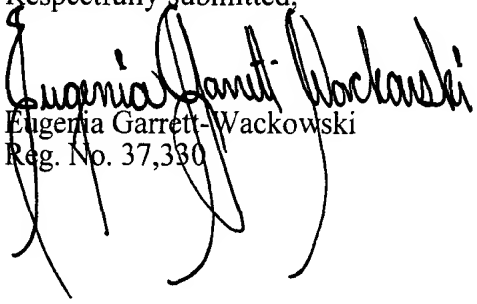
conjugate, or the use of cationic Ca^{2+} ions as an endosomal disrupting agent in the SPLP compositions. In addition, Haberland *et al.* ***teach away*** from enhancing transfection efficiency in cases where cationic liposomes are employed (*see*, page 24, column 2, Haberland *et al.*). Therefore, neither Russell *et al.* nor Haberland *et al.* supplement the deficiencies of Cullis *et al.* One of skill in the art would not have been motivated by the teachings of Russell *et al.* and Haberland *et al.* to employ Ca^{++} ions in the SPLP compositions. As such, Applicants respectfully request that the obviousness-type double patenting rejection be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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